



## Neuro-urology

# The Loss and Progressive Recovery of Voiding after Spinal Cord Interruption in Rats is Associated with Simultaneous Changes in Autonomous Contractile Bladder Activity

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### Abstract

**Background:** Autonomous contractile activity (ACA) is a well-known phenomenon in isolated bladders from different species and seems to be important in the physiology of both normal and dysfunctional voiding.

**Objective:** To determine whether ACA is changed in bladders from paraplegic rats at different periods post-spinal cord injury (post-SCI).

**Design, setting, and participants:** ACA was studied in bladders (at least six per group) from normal and paraplegic female Wistar rats at different times post-SCI (2 h, 24 h, 1 wk, and 3 wk). A group of normal rats was used as a control group. For measurements bladders were incubated in organ baths under standardised conditions.

**Measurements:** ACA was measured as pressure change, which was defined as either a transient change or a spiked change according to its characteristics. The effects of intravesical volume load and muscarinic agonists were studied.

**Results and limitations:** Following spinal cord injury (SCI) a clear evolution in ACA was observed. In bladders from SCI rats in the acute areflexive voiding phase (1 wk post-SCI), we observed decreased ACA associated with a highly increased compliance and a changed response to muscarinic agonists. ACA in bladders from SCI rats with renewed voiding reflexes (3 wk post-SCI) was increased, together with a moderately increased compliance and a (moderately) changed response to muscarinic agonists.

**Conclusions:** From these observations it is apparent that SCI leads to alterations in the behaviour and muscarinic response of ACA in the isolated bladder. These changes in ACA may play an important role in the pathophysiology of overactive bladder disease (OAB), and interacting with changed ACA might be promising in the search for newer treatments for OAB.

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## 1. Introduction

The most common functional bladder disorder is overactive bladder disease (OAB), which is a syndrome of urinary urgency with or without urge incontinence, usually with frequency and nocturia [1]. OAB with a neurological aetiology is often referred to as neurogenic detrusor overactivity (NDO). The study of the pathophysiology of NDO has mainly considered changes in bladder innervation [2], but at present there is no integrative functional bladder model that can fully explain the mechanisms underlying NDO. Recent reports have suggested that alterations in autonomous contractile bladder activity (ACA) might also play an important role in the aetiology and clinical image of functional bladder disorders [3–5]. Exaggerated and increased ACA might be partially responsible for generating nonvoiding contractions (NVC) and increased sensations of urge [3,5,6].

A frequently used animal model to study NDO is the spinal cord injured rat with a full spinal cord lesion at the thoracic vertebral levels T9–T12. Bladder function in such spinal cord injured rats has some well-described time-dependent evolutions, finally resulting in NDO. It is known that during the first 4 d post-spinal cord injury (post-SCI) rats exhibit detrusor areflexia [7,8], followed by the emergence of NVC from day 5 [7,8]. After 2–3 wk post-SCI bladder contractions gradually produce loss of urine, which results in a partial recovery of voiding function [7–9]. However, voiding is then inefficient due to detrusor-sphincter dyssynergia and is accompanied by uninhibited bladder contractions (NVC) [7,9].

In the present study we looked at ACA in bladders from SCI rats at different clinical stages in the development of NDO: during spinal shock (2 h post-SCI), during detrusor areflexia (24 h post-SCI), during the onset of NVC (1 wk post-SCI), and finally during the recovery of (dysfunctional) spontaneous voiding (3 wk post-SCI). Our aim was to investigate whether SCI induces alterations in the behaviour of ACA that could be involved in the evolution from detrusor areflexia towards NDO.

## 2. Methods

Rats used were female Wister rats (weight: 200–250 g; age: 10–14 wk; Elevage Janvier, Le Genest St Isle, France). All rats were housed according to the rules of the Institutional Animal Ethics Committee, and appropriate ethical approval was obtained for these experiments. Rats were sacrificed using CO<sub>2</sub> overdose at four different time periods after SCI: 2 h, 24 h, 1 wk, and 3 wk.

### 2.1. Procedures on animals

SCI was performed under 3.5% isoflurane anaesthesia (Isoba, Shering-Plough, Uxbridge, UK) and prophylaxis with antibiotics (0.6 ml ampicillin [100 mg/ml], Duphamox, Norbrook Laboratories, Newry, Northern Ireland). The procedure involved a laminectomy at the vertebral level T8–T9, interrupting the spinal cord at T9 and closing the surgical wound. A cr  de-manoeuvre—emptying the bladder by application of smooth abdominal pressure—was performed twice daily, and antibiotics were given every 2 d during the first week. After surgery,

attention was given to thermoregulation and to prevention and treatment of decubitus lesions.

Volume of urine in bladder was estimated by measuring the amount of urine collected by cr  de manoeuvres in rats from all groups (at least 10 animals per group). This urine collection was always performed in the morning to exclude diurnal variability in urine production.

### 2.2. Pressure measurements

Animals were sacrificed and a laparotomy was performed. A small catheter (22 gauge, Insyte-W, Becton-Dickinson, Aalst, Belgium) was inserted in the urethra for transurethral pressure measurement and secured with a ligature. The bladder was then excised entirely after ligation of both ureters. A strict time schedule was maintained following bladder excision. The bladder was excised at minute 0 and directly placed in cold Krebs (NaCl 118 mmol/l, KCl 4.6 mmol/l, CaCl<sub>2</sub> 2.5 mmol, MgSO<sub>4</sub> 1.2 mmol/l, KH<sub>2</sub>PO<sub>4</sub> 1.2 mmol/l, NaHCO<sub>3</sub> 25 mmol/l, glucose 11 mmol/l, ultra pure water; 4 °C). The bladder was then placed in a heated organ bath at minute 5, and the first measurements were made approximately 30 min later. The incubation fluid was Krebs solution. This was constantly gassed with a mixture of oxygen (95%) and CO<sub>2</sub> (5%), and the temperature was maintained at a mean of 36 °C with a pH of 7.40. The intraurethral catheter was connected via a three-way tap to a volume pump (Harvard Apparatus, Holliston, MA, USA) to allow increase of intravesical volume and to a DTX plus pressure transducer (Becton-Dickinson, Aalst, Belgium; zero offset 25 mmHg, natural frequency 100 Hz), from which the output was amplified and recorded using the Windaq system (Dataq Instruments, Akron, OH, USA). Pressure transducers were calibrated before every experiment.

Each experiment consisted of two parts. First the bladder capacity was established. In the absence of a formal definition of capacity in the isolated bladder, we used the definition of hydromechanical capacity as described previously [3], although some adaptations were made. Bladders were allowed to adapt their volume spontaneously at 36 °C, and the intravesical baseline pressure at that volume was set at 0 cm H<sub>2</sub>O. Successively, bladders were filled at 20 µl/min, and the volume at which baseline pressure had increased with a constant pressure of 0.5 cm H<sub>2</sub>O was considered as hydromechanical capacity (580 ± 12 µl in the control group vs 1370 ± 36 µl at 3 wk post-SCI). In volume-load experiments we let bladders spontaneously adapt their volume to 36 °C, and successively we started bladder filling. This different protocol was used to obtain fair compliance data. Once bladder capacity was established, the experimental measurements were performed. For volume load, pressures were measured during 45 min with 15 min of resting status, 15 min of filling status at a rate of 20 µl/min (with Krebs solution), and 15 min of resting status after the filling phase. For muscarinic responses, cumulative doses were added every 5 min (carbachol 10<sup>-8.5</sup>–10<sup>-6</sup> M, arecaidine but-2-ynyl ester tosylate (ABET) 10<sup>-9.5</sup>–10<sup>-7</sup> M). All drugs were added in volumes of 100 µl from a stock solution. Bladders were weighed after each experiment. Each experiment was performed on at least six whole bladders per group.

### 2.3. Drugs

Drugs used were the nonselective muscarinic agonist carbachol (Sigma, Bornem, Belgium) and the weak partial M<sub>2</sub>-agonist ABET (pD<sub>2</sub> for M<sub>2</sub> = 8.33 and pD<sub>3</sub> for M<sub>3</sub> = 7.67 [10]; Tocris, Bristol, UK). All drugs were dissolved in purified water, made as stock solutions, and stored at 4 °C.

### 2.4. Analytic definitions and statistical analysis

All analyses were performed during equal time periods. ACA was measured as change in intravesical pressure, and for statistical comparison pressure values were normalised to bladder weight.

Consistent with previous publications we divided intravesical pressure change into two groups [3,11]. Transients were defined as pressure changes of at least 0.5 cm H<sub>2</sub>O, lasting between 5 s and 30 s, and ending when the pressure level had fallen to 25% of its original height. Spikes were defined as pressure changes of at least 0.1 cm H<sub>2</sub>O, lasting between 0 s and 3 s, and ending when the pressure level had returned to the initial baseline. Compliance was calculated using the following formula: volume load (ml)/increase in baseline pressure normalised to bladder weight ( $\Delta$  cm H<sub>2</sub>O/g). All cholinergic effects on ACA were calculated relative to baseline values and expressed as increases in amplitude or baseline. Cholinergic concentration–response curves are graphically presented by fitting sigmoidal curves to the experimental data (Graphpad Prism software, San Diego, USA). Potencies are expressed as pD<sub>2</sub> values (=  $-\log$  EC<sub>50</sub>).

Quantitative values are shown as the mean plus or minus standard error. Statistics were performed with Statistica 7.1 (Statsoft, Tulsa, OK, USA). An analysis-of-variance (ANOVA) test was used to compare different groups, followed by a Fisher post hoc test.

### 2.5. Quantitative polymerase chain reaction

Dissected bladders were incubated in Ca<sup>2+</sup>/Mg<sup>2+</sup>-free medium (Invitrogen, Merelbeke, Belgium). Instant quantitative polymerase chain reactions (QPCR) were performed in triplicate using cDNA generated from whole bladder mRNAs ( $n = 4$  per group) and specific rat M<sub>2</sub> (muscarinic receptor, Rn02532311\_s1; Applied Biosystems) and M<sub>3</sub> (Rn01470216\_m1; Applied Biosystems) TaqMan assays. The housekeeping gene  $\beta$ -actin (Applied Biosystems), which remained constant post-SCI (data not shown), was used as an endogenous control to normalise M<sub>2</sub>/M<sub>3</sub> mRNA. Data (mean plus or minus SE) are shown as an  $x$ -fold expression of detected mRNA normalised to M<sub>3</sub> mRNA in bladders of adult rats, which was used as a calibrator for the comparative critical threshold ( $\Delta\Delta$ Ct) analysis.

## 3. Results

### 3.1. Bladder weight and bladder volume post-spinal cord injury (Fig. 1)

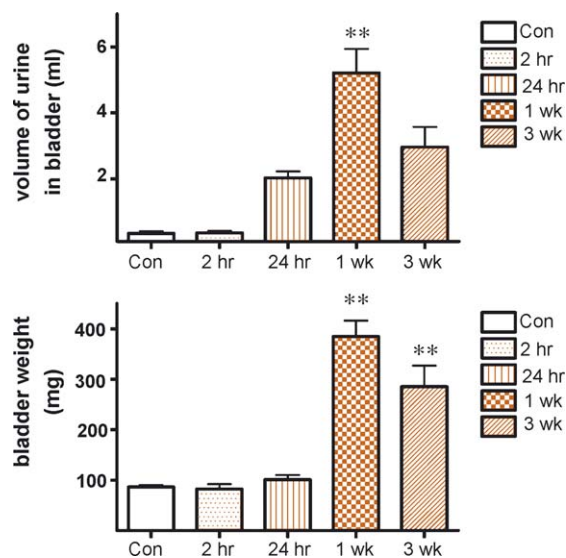
Volume of urine in bladder showed a steady increase post-SCI, reaching its maximum at 1 wk post-SCI ( $p < 0.005$ ). Bladder weight was significantly increased at 1 wk and 3 wk post-SCI ( $p < 0.005$ ). (Voiding behaviour of rats post-SCI is shown in Supplementary Fig. 1).

### 3.2. Behaviour of autonomous contractile activity post-spinal cord injury (Fig. 2)

The frequency of transients decreased progressively post-SCI, reaching its lowest value at 1 wk post-SCI ( $p < 0.005$ ). The amplitude of transients globally increased at 2 h post-SCI ( $p < 0.005$ ). From 24 h post-SCI onward, the duration of transients became significantly longer ( $p < 0.005$ ). The frequency of spikes decreased until 1 wk post-SCI ( $p < 0.005$ ); at 3 wk post-SCI the frequency of spikes became higher compared to controls ( $p < 0.05$ ).

### 3.3. Volume-induced changes in autonomous contractile activity post-spinal cord injury (Fig. 3)

Compliance increased post-SCI, reaching its highest value at 1 wk post-SCI ( $p < 0.005$ ). The volume-induced increase in

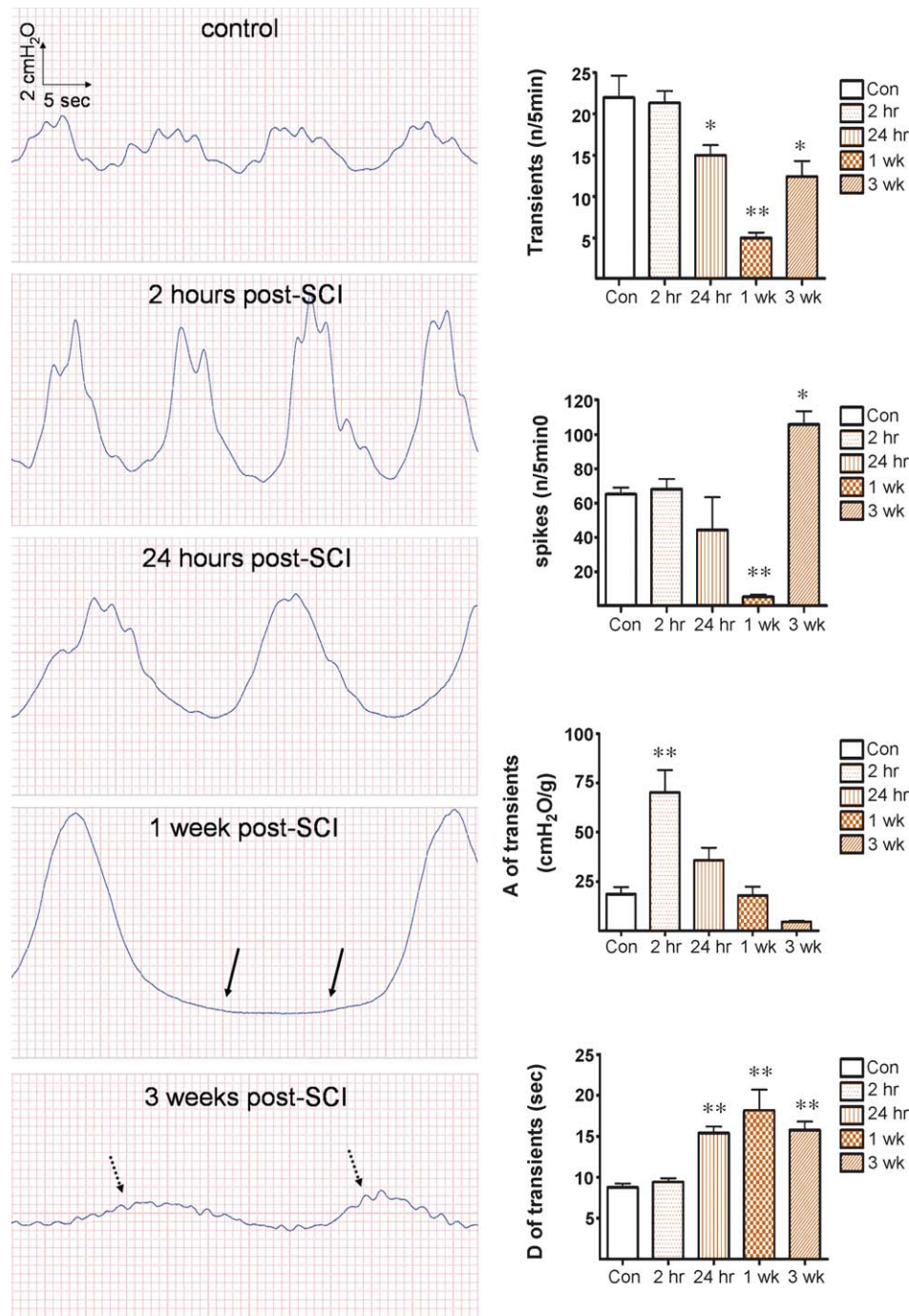


**Fig. 1** – Graphs illustrating bladder weight and volume of urine in bladder at different periods post-spinal cord injury (post-SCI). Maximal values of both parameters were observed around 1 wk post-SCI. An asterisk (\*) indicates significance ( $* p < 0.05$ ;  $** p < 0.005$ ). Significance values in bladders post-SCI are always compared to normal bladder values. Only the most relevant significance levels are indicated. Data are means plus or minus SE.

contractile amplitude of transients decreased significantly only at 2 h post-SCI ( $p < 0.05$ ). The volume-induced increase in frequency of transients was impaired at periods later than 24 h post-SCI, resulting in larger statistical differences between different groups ( $p < 0.005$ ). Volume had no obvious effects on frequency of spikes in all of the studied groups.

### 3.4. Response to muscarinic agonists post-spinal cord injury (Figs. 4 and 5)

Carbachol induced an increase in amplitude of transients and baselines in all groups, which was significantly more pronounced in bladders 2 h post-SCI ( $p < 0.005$ ). PD<sub>2</sub>-values for carbachol effects on amplitude and baseline were significantly increased between 2 h post-SCI and 1 wk post-SCI compared to controls; at 3 wk post-SCI pD<sub>2</sub> values were again similar to controls [for amplitude:  $p < 0.05$ ; mean plus or minus SE (group):  $6.5 \pm 0.4$  (control),  $6.8 \pm 0.2$  (2 h),  $7.0 \pm 0.2$  (24 h),  $8.2 \pm 2.5$  (1 wk), and  $6.8 \pm 0.3$  (3 wk); for baseline:  $p < 0.05$ ; mean plus or minus SE (group):  $7.2 \pm 0.2$  (control),  $7.6 \pm 0.2$  (2 h),  $7.7 \pm 0.3$  (24 h),  $7.6 \pm 0.1$  (1 wk), and  $7.3 \pm 0.1$  (3 wk)]. The weak M<sub>2</sub>-selective drug ABET induced significantly larger increases in contractile amplitude in bladders 1 wk post-SCI compared to other groups post-SCI ( $p < 0.005$ ) and compared to controls ( $p < 0.005$ ). In groups 24 h post-SCI and 3 wk post-SCI this increase was less pronounced but mostly still significant compared to controls ( $p < 0.05$ ); in controls the increase in contractile amplitude to ABET was negligible (Figs. 4 and 5). PD<sub>2</sub>-values for ABET effects on amplitude were decreased at 1 wk post-SCI and 3 wk post-SCI compared to controls, although not significantly [mean plus or minus SE (group):  $9.3 \pm 0.6$  (control),  $9.3 \pm 0.7$  (2 h),  $9.1 \pm 0.5$  (24 h),  $8.4 \pm 0.4$  (1wk) and  $7.7 \pm 0.3$  (3 wk)]. An increase in the contractile baseline due to ABET became

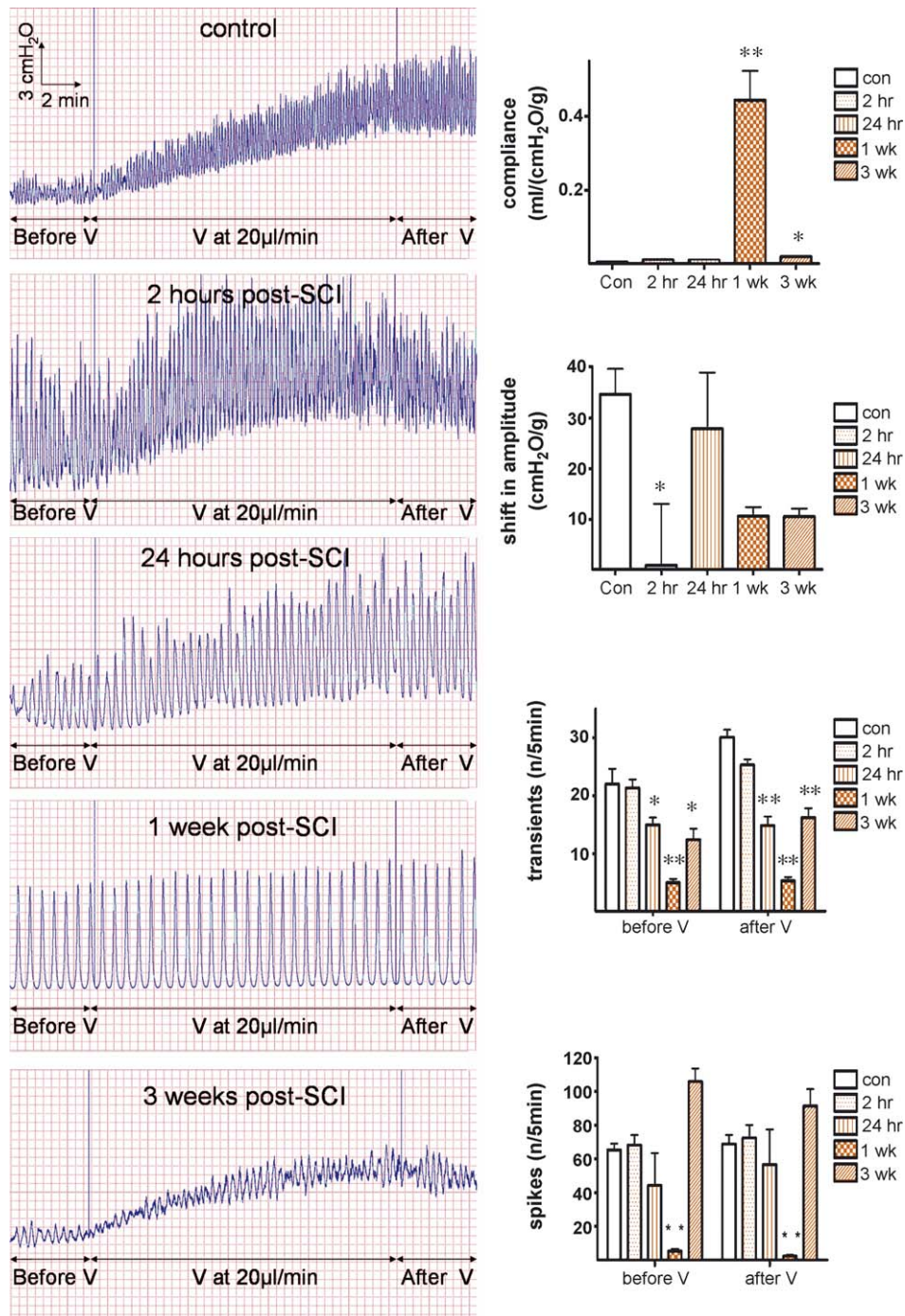


**Fig. 2 – Autonomic contractile activity (ACA) at different periods post–spinal cord injury (post-SCI).** Left panels: pattern of ACA at different periods post-SCI: control, 2 h post-SCI, 24 h post-SCI, 1 wk post-SCI, and 3 wk post-SCI. The contractile frequency of ACA is significantly decreased at 1 wk post-SCI (long intervals, full arrows). At 3 wk post-SCI, ACA becomes irregular and exaggerated (abundant spikes, broken arrows). Pressure and time scales are the same in all panels. Right panels: Graphs illustrating evolution of different contractile parameters post-SCI: frequency of transients, frequency of spikes, amplitude (A) and duration (D) of transients. An asterisk (\*) indicates significance (\*  $p < 0.05$ ; \*\*  $p < 0.005$ ). Significance levels in bladders post-SCI are always compared to normal bladder values. Only the most relevant significances are indicated. Data are means plus or minus SE.

apparent at higher drug concentrations, most pronounced (but without significance) in bladders at 1 wk post-SCI. Potency for ABET effects on baseline decreased post-SCI compared to controls; therefore accurate pD<sub>2</sub> values for post-SCI groups could not be generated [mean plus or minus SE:  $8.2 \pm 0.4$  (control)].

**3.5. Quantitative polymerase chain reaction data on M2 and M3 in whole bladders post–spinal cord injury (Fig. 6)**

QPCR showed a significant increase of M<sub>2</sub> mRNA in bladders at 3 wk post-SCI compared to controls ( $p < 0.05$ ), but no changes were observed at earlier stages post-SCI. The

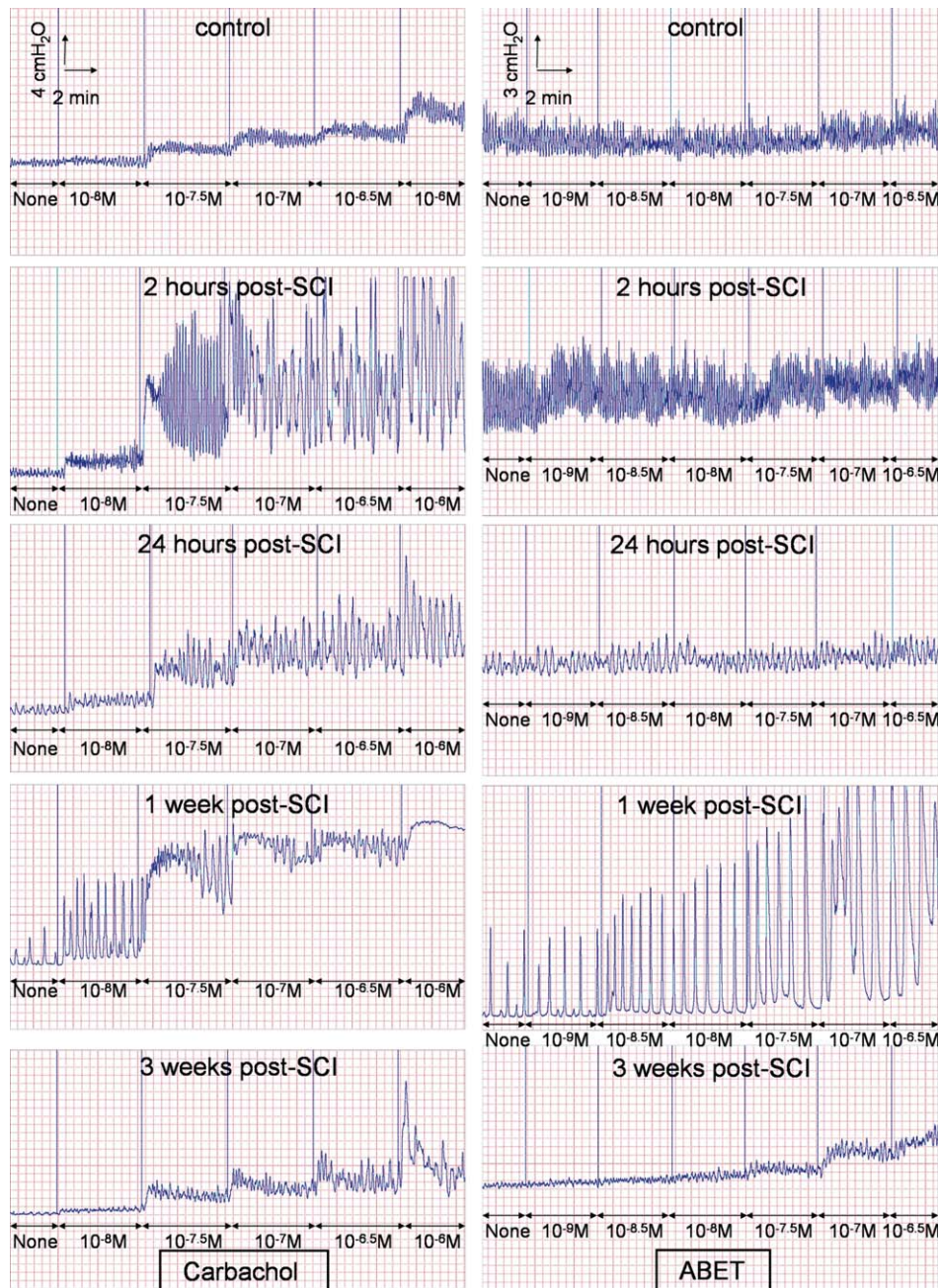


**Fig. 3** – Effects of intravesical volume on autonomous contractile activity (ACA) at different periods post–spinal cord injury (post-SCI). Left panels: pattern of volume-induced effects (20  $\mu$ l/min) on ACA at different periods post-SCI: control, 2 h post-SCI, 24 h post-SCI, 1 wk post-SCI, and 3 wk post-SCI. Pressure and time scales are the same in all panels. Right panels: Graphs illustrating volume-induced effects on ACA at different periods post-SCI: effects of intravesical volume load (20  $\mu$ l/min) on compliance, on amplitude and frequency of transients, and on spikes. An asterisk (\*) indicates significance (\*  $p < 0.05$ ; \*\*  $p < 0.005$ ). Significance values in bladders post-SCI were always compared to normal bladder values. Only the most relevant significances are indicated. Data are means plus or minus SE.

amount of  $M_3$  mRNA had significantly decreased in bladders at 24 h post-SCI ( $p < 0.005$ ), but not in any of the other post-SCI groups. Due to these changes in muscarinic mRNA expression, the  $M_2/M_3$  ratio had significantly increased in bladders at 24 h post-SCI ( $p < 0.05$ ) and at 3 wk post-SCI ( $p < 0.005$ ).

#### 4. Discussion

Most research on mechanisms underlying NDO has focussed on changes in the neural pathways [2,7]. It is, however, becoming more and more clear that changes in ACA might also contribute to the clinical image of NDO.



**Fig. 4 – Original records show effects of cumulative doses of muscarinic agonists [carbachol and arecaidine but-2-nyl ester tosylate (ABET)] on autonomous contractile activity (ACA) in all post–spinal cord injury (post-SCI) groups. Pressure and time scales used in left and right panels are the same.**

Early evidence for this thesis came from data on obstructed [5] and neurogenic bladders [3,6,12], and the present study further supports such theory. The decreased ACA (in frequency of transients and spikes) at 1 wk post-SCI might play a role in the clinical manifestation of urine retention, while the unstable ACA (increased frequency of spikes) at 3 wk post-SCI could be partially responsible for the sensation of urgency and the emergence of NDO. The observed ACA at 7 d post-SCI might be due to a chronic absence of interaction with neural pathways, while neural sprouting post-SCI might induce and/or facilitate the increased and unstable ACA as seen at 3 wk post-SCI. Bladder hypertrophy might

also be an aetiological factor in the changing ACA post-SCI, otherwise alterations in ACA might facilitate the emergence of axonal sprouting and the recovery of voiding. As suggested by others, increased ACA could be an endogenous mechanism to increase afferent signalling in an attempt to re-establish voiding reflexes [6,13].

It is known that bladder-wall compliance may decrease in bladders from chronic SCI patients [14,15]. In SCI rats it was demonstrated with *in vitro* [16] and *in vivo* [7] experiments that 3 wk post-SCI there is a significant increase in bladder compliance. In the present series of experiments we confirmed this increase in bladder com-

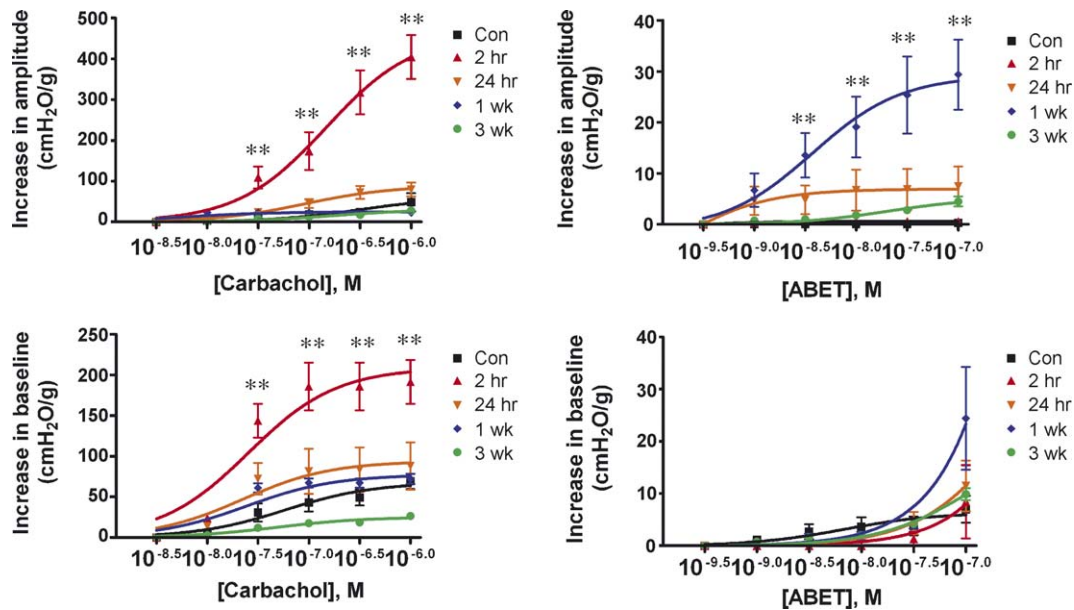


Fig. 5 – Graphs illustrate effects of muscarinic agonists [carbachol and arecaidine but-2-ynyl ester tosylate (ABET)] on the amplitude of transients and contractile baselines in all groups post-spinal cord injury (post-SCI). An asterisk (\*) indicates significance (\*  $p < 0.05$ ; \*\*  $p < 0.005$ ). Significance values in bladders post-SCI were always compared to normal bladder values. Only the most relevant significances are indicated. Data are means plus or minus SE.

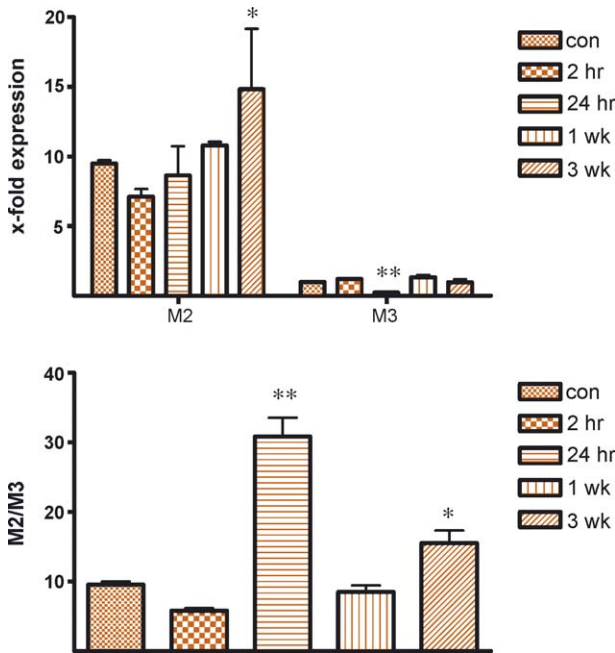


Fig. 6 – Quantitative polymerase chain reaction (QPCR) analysis of M<sub>2</sub> and M<sub>3</sub> mRNA expression post-spinal cord injury (post-SCI) in rat bladder. Upper graph: Relative quantification of M<sub>2</sub> and M<sub>3</sub> mRNA expression in full bladders at 2 h, 24 h, 1 wk, and 3 wk post-SCI compared to controls. One endogenous control,  $\beta$ -actin, was used for normalisation. Data are shown as x-fold expressions of detected mRNA normalised to M<sub>3</sub> mRNA in bladders of adult rats, which was used as a calibrator for the comparative critical threshold ( $\Delta\Delta Ct$ ) analysis. Data are from four to six samples for each group. Data are means plus or minus SE. An asterisk (\*) indicates significance (\*  $p < 0.05$ ; \*\*  $p < 0.005$ ). Lower graph: ratio between M<sub>2</sub> and M<sub>3</sub> mRNA levels presented in the upper graph. Data are means plus or minus SE. An asterisk (\*) indicates significance (\*  $p < 0.05$  and \*\*  $p < 0.005$ ).

pliance through a different in vitro experimental approach. However, our observations might begin to explain the discrepancies between increased compliance in bladders from early SCI rats and the increased stiffness of bladders from chronic SCI patients. The different voiding behaviour in both groups could subject the structures of the bladder wall to different mechanical environments: sustained distension in areflexive (and beginning overactive) bladders [7,16] as opposed to increased work and dynamic pressure load in long-term overactive bladders [14,15], which might then result in increased and decreased compliance respectively. The observation in this study that in bladders from rats with beginning NDO compliance (3 wk post-SCI) had already decreased seems to affirm this idea.

Acetylcholine (Ach) is an important efferent neurotransmitter in bladder and M<sub>3</sub> the muscarinic receptor subtype that directly mediates contractions (for review see Hegde [17]). Various changes in cholinergic bladder physiology have been reported post-SCI in rat [2,8] and in the present study we found evidence for SCI-related increased sensitivity to muscarinic agonists.

It is remarkable that, with the two agonists used, the increased contractile response of ACA occurred at different times: carbachol had an increased response only at 2 h post-SCI, while the increased effects of ABET were noticed between 24 h and 3 wk post-SCI, with a peak at 1 wk post-SCI. The increased carbachol effects at 2 h post-SCI are possibly due to acute denervation supersensitivity as described by others [18,19]. The mechanisms underlying the ABET effects remain mainly unclear with respect to the present data, although the weak M<sub>2</sub>-preference might be involved. There are many reports on changed M<sub>2</sub>-signalling in the neurogenic bladder. Despite its predominance in bladder [20], M<sub>2</sub> normally only has indirect effects on

M<sub>3</sub>-signalling [21]. In decentralised rat bladders an enhanced and direct contractile role for M<sub>2</sub> has been reported [19]. Similar results were found in obstructed [22] and neurogenic [3,23] rat bladders. The weak M<sub>2</sub>-preference of ABET might then be partially responsible for the increased contractile effects of ABET on ACA in bladders post-SCI, if these bladders indeed have an enhanced M<sub>2</sub>-function. To confirm this hypothesis, selective antagonist studies should be performed in future experiments. In humans such changed M<sub>2</sub>-function might also develop in NDO, although there are conflicting reports on this topic. Some authors reported that bladder tissue from patients suffering from NDO showed a shift from M<sub>3</sub> towards M<sub>2</sub> in direct mediation of bladder contractions [24], whereas others found that M<sub>3</sub> was still responsible for muscarinic-induced contractions in neurogenic bladders with no changes in M<sub>2</sub> signalling [25].

Further study of cholinergic changes post-SCI was performed at the mRNA level. We found a significant increase of M<sub>2</sub> mRNA at 3 wk post-SCI and a significant decrease of M<sub>3</sub> mRNA at 24 h post-SCI. The observed increase in M<sub>2</sub> mRNA is likely to correlate with an elevated expression of M<sub>2</sub> receptors since an increased density of M<sub>2</sub> protein has been reported in decentralised [19], obstructed [22], and neurogenic [23] rat bladders. The behaviour of M<sub>3</sub> mRNA in bladders post-SCI seems to correlate less with the expression of M<sub>3</sub> protein since in hypertrophied [22] and (spontaneous voiding) neurogenic [23] rat bladders a decrease of M<sub>3</sub> protein amount has been reported. Similar noncorrelating findings have been reported before [26] and suggest differences in the cascade from mRNA to protein between M<sub>2</sub> and M<sub>3</sub>. The observed changes at the mRNA level are likely to be involved in the increased sensitivity for muscarinic agonists post-SCI, but the exact mechanisms remain unclear from the present study. Changes in muscarinic downstream signalling might also be involved, as suggested by others [27].

## 5. Conclusion

In conclusion we have presented further evidence for changed ACA in the neurogenic bladder. Besides being essential for the aetiology and symptoms of NDO, changed ACA in functional bladder disorders might also offer new therapeutic approaches. Interacting on local targets could be promising in new treatments for NDO, due to the possible advantage of fewer side-effects and less disturbance of normal voiding pathways. Based on the present data, it might then be interesting to further explore the mechanisms and receptor subtypes involved in the observed increased sensitivity to muscarinic agonists.

**Author contributions:** Thomas Gevaert had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Study concept and design:** Gevaert, De Ridder, van Leuven.

**Acquisition of data:** Gevaert, van Leuven, Owsianik, Everaerts.

**Analysis and interpretation of data:** Gevaert, Everaerts.

**Drafting of the manuscript:** Gevaert, Hutchings, De Ridder.

**Critical revision of the manuscript for important intellectual content:** De Ridder, Nilius, Hutchings.

**Statistical analysis:** Owsianik, Gevaert.

**Obtaining funding:** De Ridder, Nilius, Gevaert.

**Administrative, technical, or material support:** Owsianik.

**Supervision:** De Ridder, Nilius.

**Other (specify):** None.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.eururo.2008.06.064.

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**Editorial Comment on: The Loss and Progressive Recovery of Voiding after Spinal Cord Interruption in Rats is Associated with Simultaneous Changes in Autonomous Contractile Bladder Activity**

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This study is unique and interesting because a very rapid, in vivo experiment just after sacrifice with excised whole urinary bladder had been done. The authors strongly pointed out that the autonomous contractile activity (ACA) is very important for the pathophysiology of neurogenic detrusor overactivity (NDO) in spinal cord injured (SCI) rats. Exaggerated and increased ACA might be partially responsible for generating nonvoiding contractions (NVC) and increased sensation of urge [1–3]. In both acute SCI with areflexic stage and renewed voiding phase (3 wk after SCI), M2 receptor might be rather strongly related to ACA. Hence, the possibility of M2 receptor selective antagonist or agonist in some stage of SCI might be useful, as previously published [4]. For the guarantee of pharmacological evaluation, mRNA expression or protein expression studies are important and should be done.

In these experiments, changes in local bladder function could be seen using excised bladder without the effect of central innervations. In addition, the implication of muscarinic receptor subtypes in the change of ACA might

be evaluated. However, recent papers suggested that changes in both afferent innervations and urothelial function might be very important [5]. In these experimental methods, afferent nerve function, local reflex arc, and urothelial function cannot be evaluated. In addition to these shortcomings, muscarinic receptor subtypes could not be fully evaluated.

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